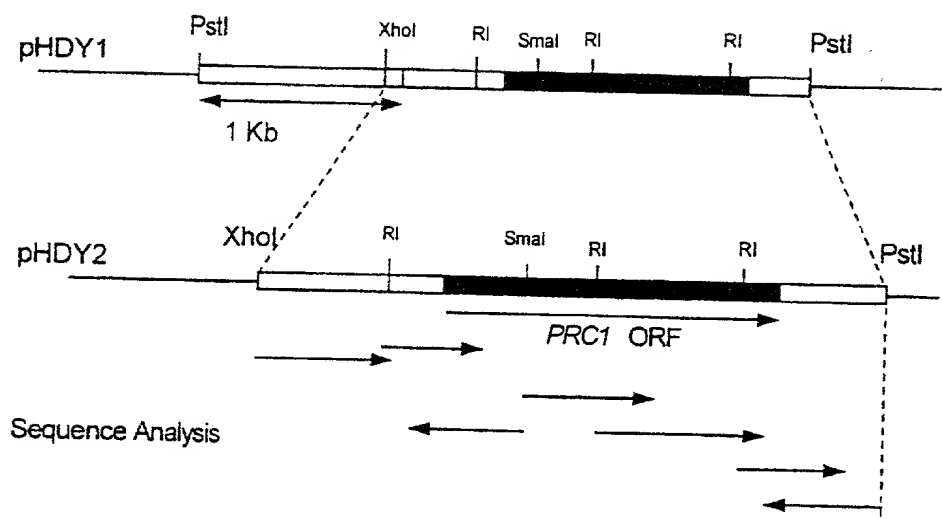
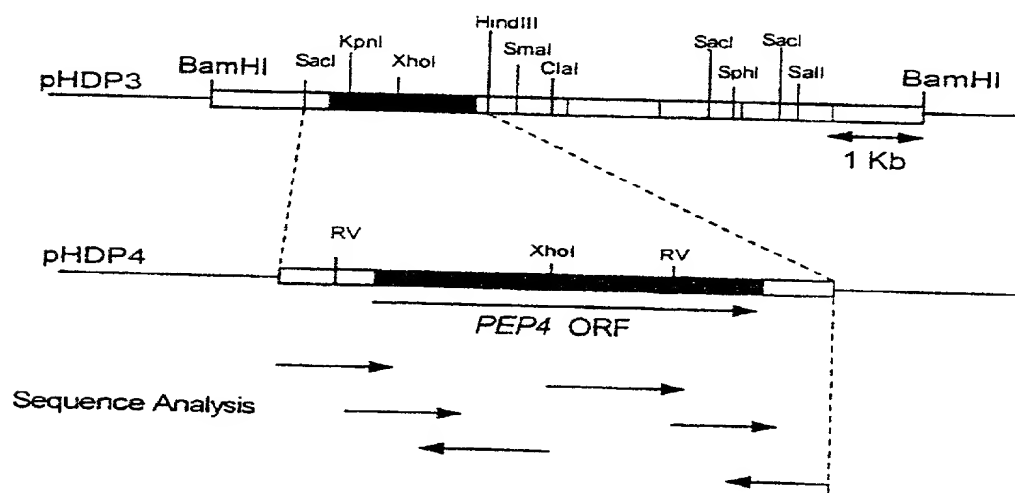


1/13

FIG. 1**FIG. 2**

2/13

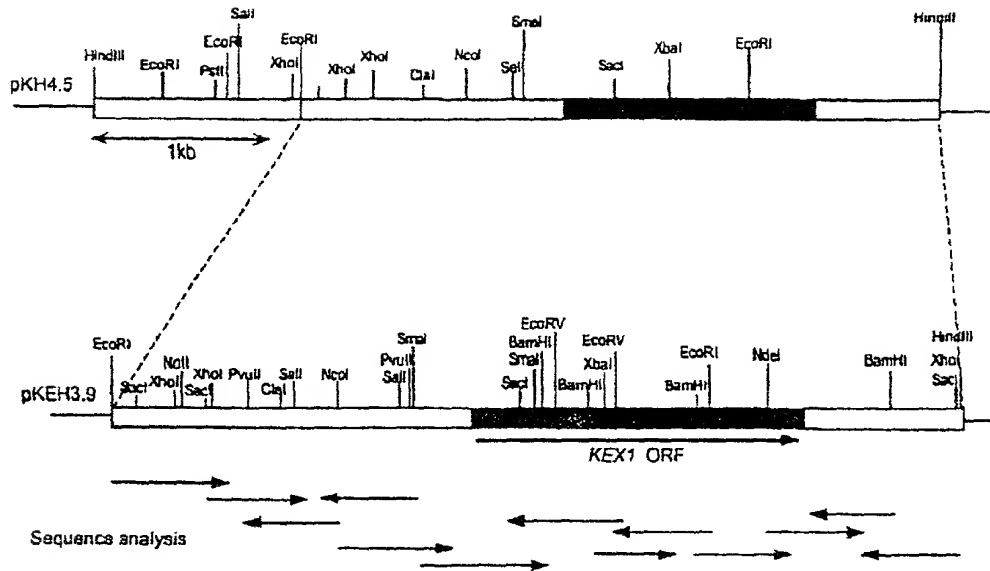
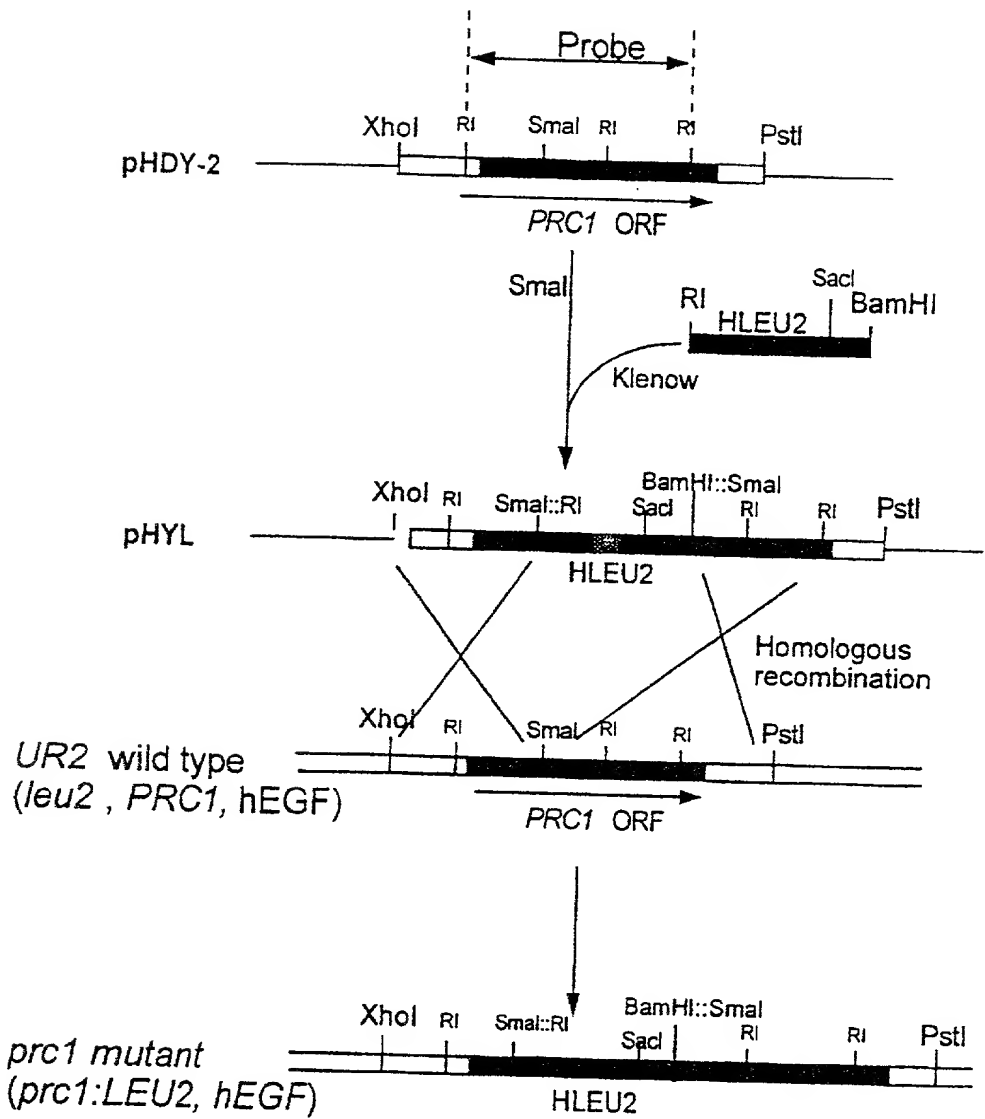
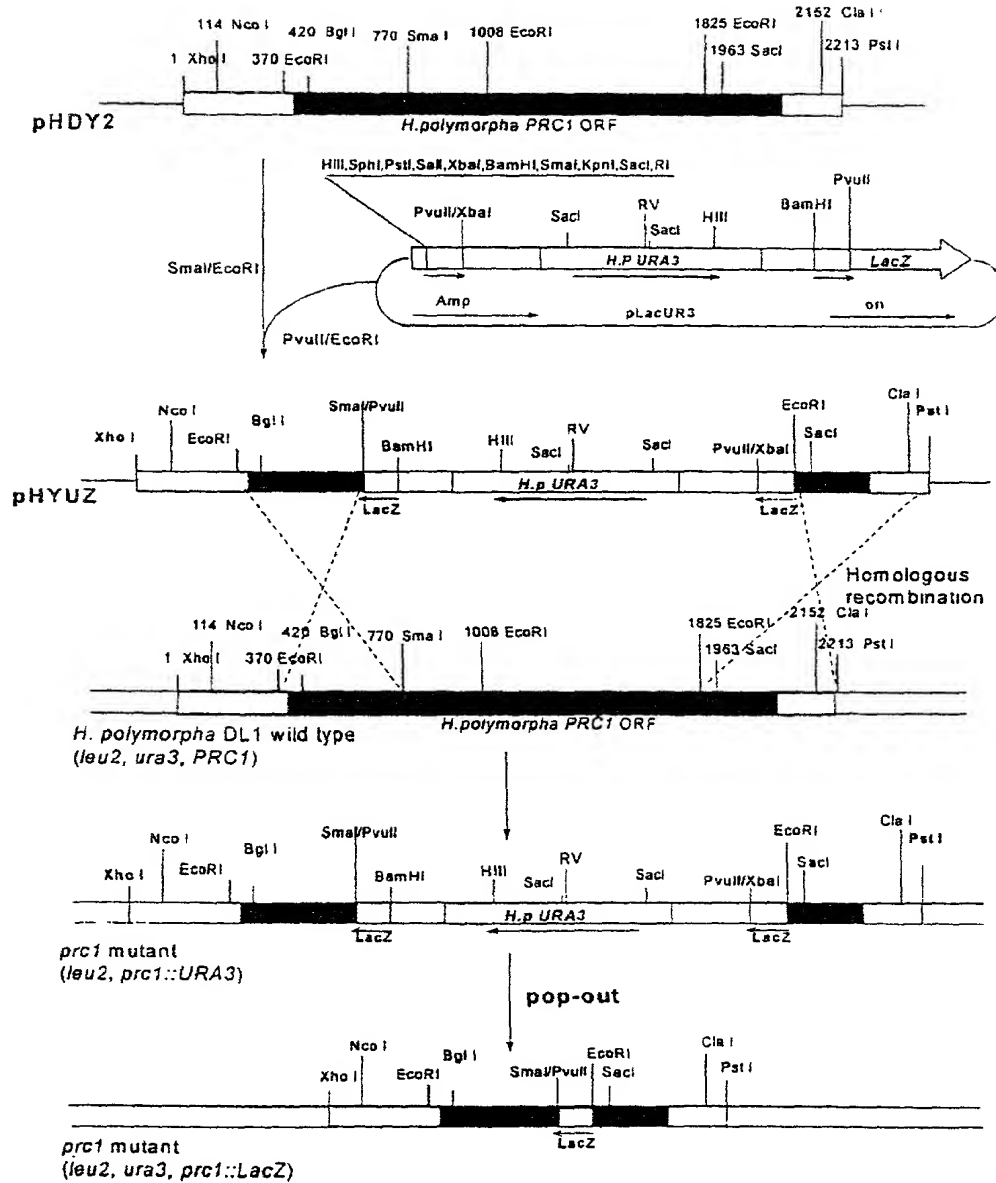
FIG. 3

FIG. 4

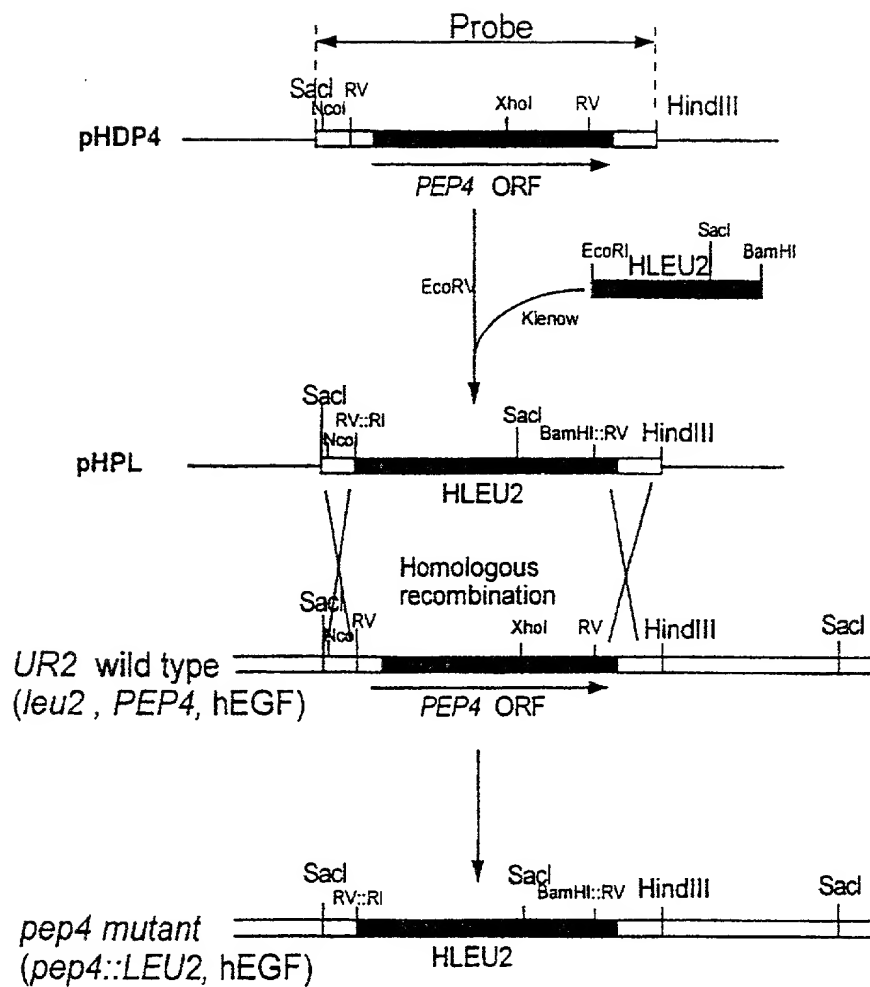


4/13

FIG. 5



5/13

FIG. 6

6/13

FIG. 7

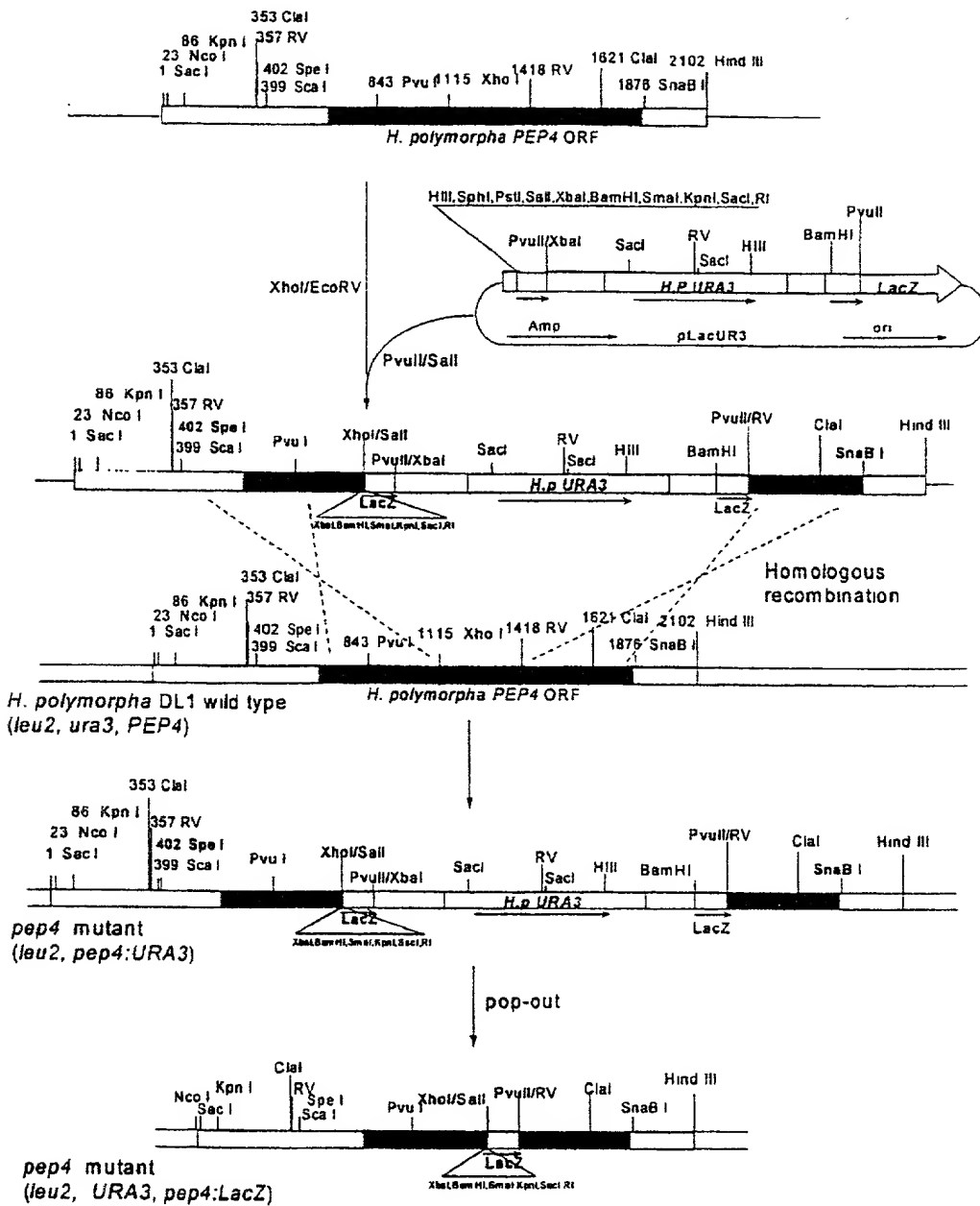


Diagram illustrating the construction of a *kex1::LacZ* reporter construct and its use in generating a *kex1::LacZ* mutant.

The top panel shows the *KEX1* ORF (Open Reading Frame) with various restriction sites (EcoRI, XhoI, NotI, PvuII, SalI, NcoI, PstI, SmaI, BamHI, XbaI, EcoRV, HindIII, SacI, KpnI, SphI, PfuI, MluI, XbaI, PstI, SmaI, KpnI, SacI, R). A probe region is indicated.

A *Smal/EcoRI* fragment is identified and inserted into a *pLacURA3* plasmid, creating a *pLacURA3* construct containing the *KEX1* ORF.

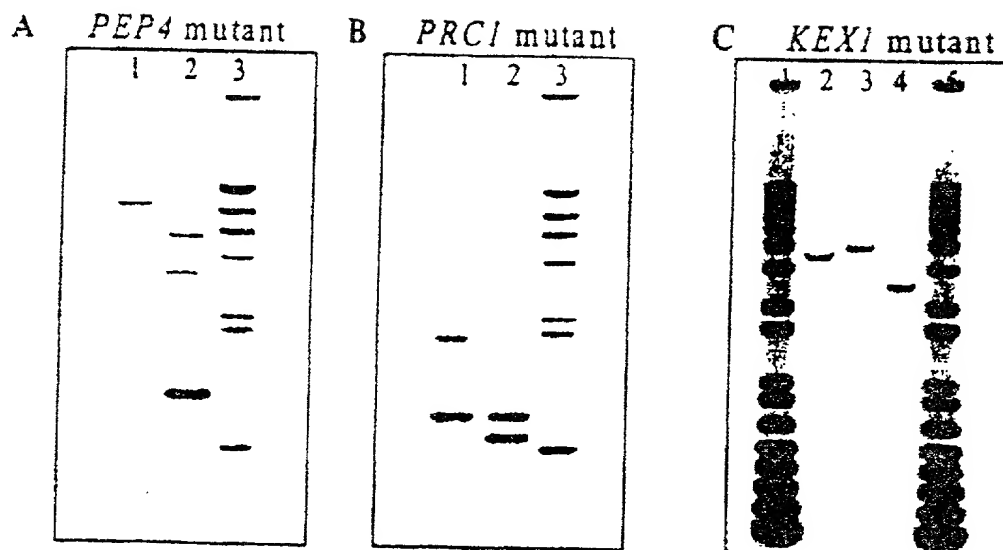
This construct is integrated into the *KEX1* locus via homologous recombination, replacing a portion of the *KEX1* ORF with the *LacZ* gene.

The resulting construct is used to generate a *kex1::LacZ* mutant (labeled "x1 mutant" and "u2, ura3, kex1::LacZ").

The *kex1::LacZ* mutant is then used to generate a *kex1::LacZ* pop-out mutant (labeled "pop-out").

kex1 mutant
(*leu2, ura3, kex1::LacZ*)

8/13

FIG. 9

A. Lane 1 : *H. polymorpha* UR2/*EcoRV*
 Lane 2 : *pep4* mutant/*EcoRV*
 Lane 3 : DIG- λ /*HindIII* size marker
 probe : DIG labeled pHDP4/*SacI*/*HindIII* 2kb fragment

B. Lane 1 : *H. polymorpha* UR2/*EcoRI*
 Lane 2 : *prc1* mutant/*EcoRI*
 Lane 3 : DIG- λ /*HindIII* size marker
 probe : DIG labeled pHDY2/*EcoRI* 1.5kb fragment

C. Lane 1,5 :DIG-1 kb size marker
 Lane 2 : *H. polymorpha* DL1/*XhoI*
 Lane 3 : *kex1* mutant/*XhoI*
 Lane 4 : URA3 gene pop-out /*XhoI*
 probe : DIG labeled pKH4.5/*XhoI* 3.5kb fragment

9/13

FIG. 10

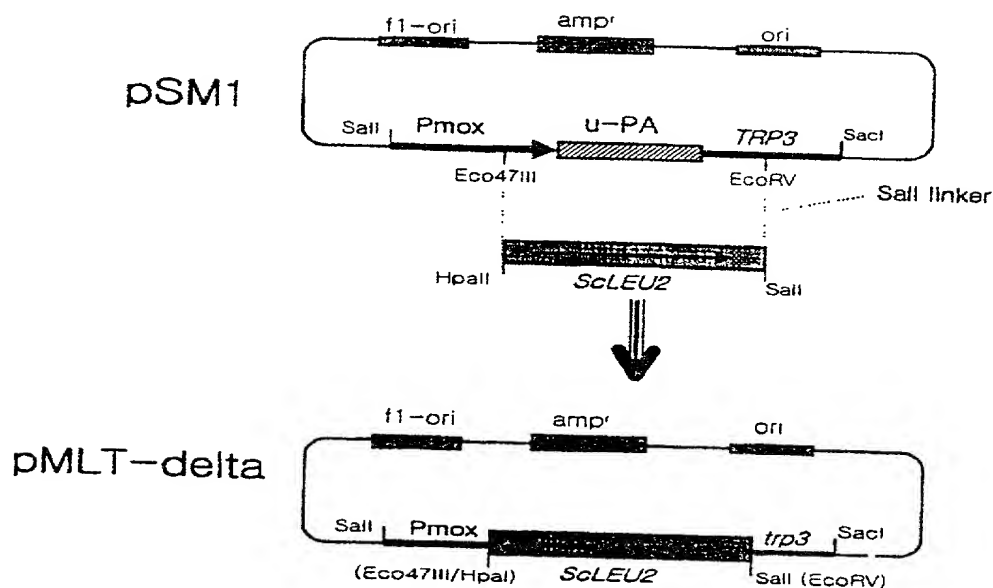
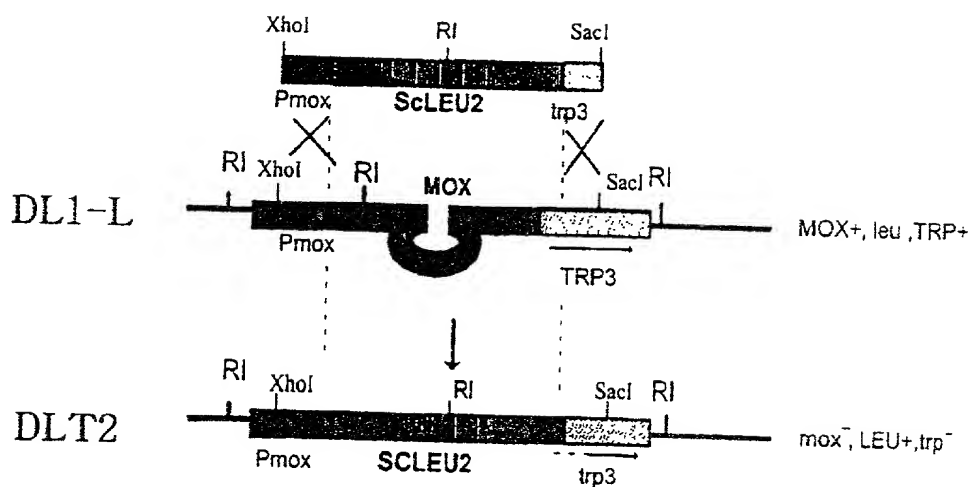


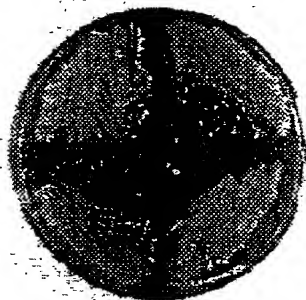
FIG. 11



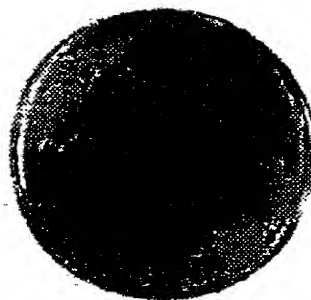
10/13

FIG. 12

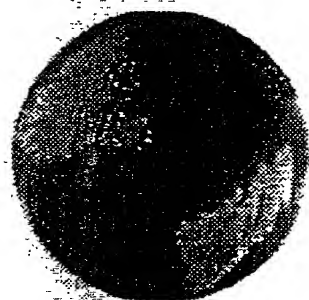
A. 2% Glucose + 0.67% YNB
+ LEU + TRP



B. 0.5% Methanol + 0.67% YNB
+ LEU + TRP



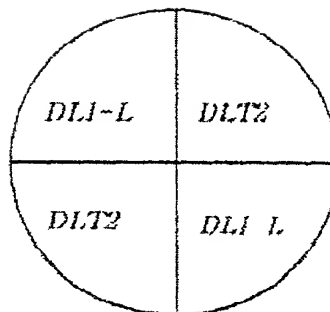
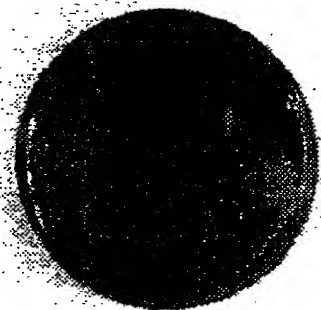
C. 2% Glucose + 0.67% YNB
+ LEU



D. 2% Glucose + 0.67% YNB
+ TRP



E. 2% Glucose + 2% Peptone
+ 1% Yeast extract



T04T1" 25TE6660

11/13

FIG. 13a

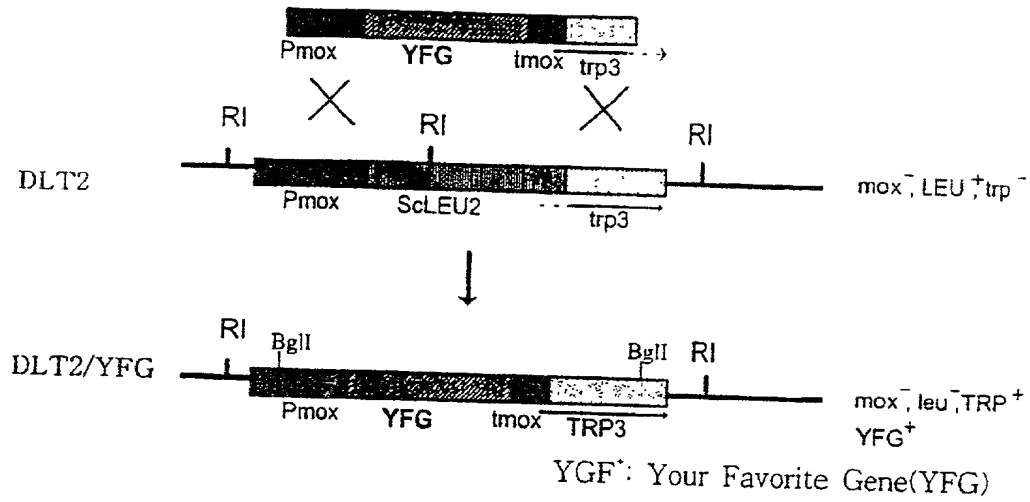
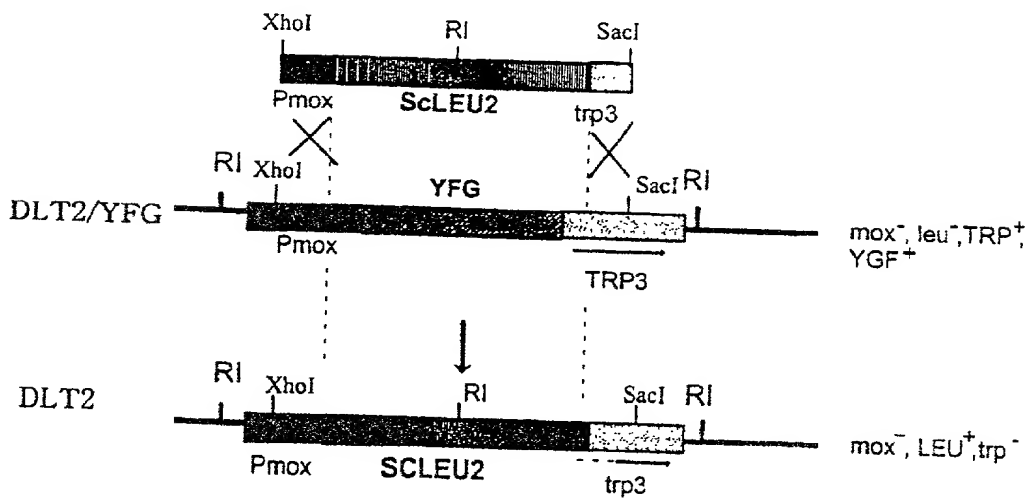
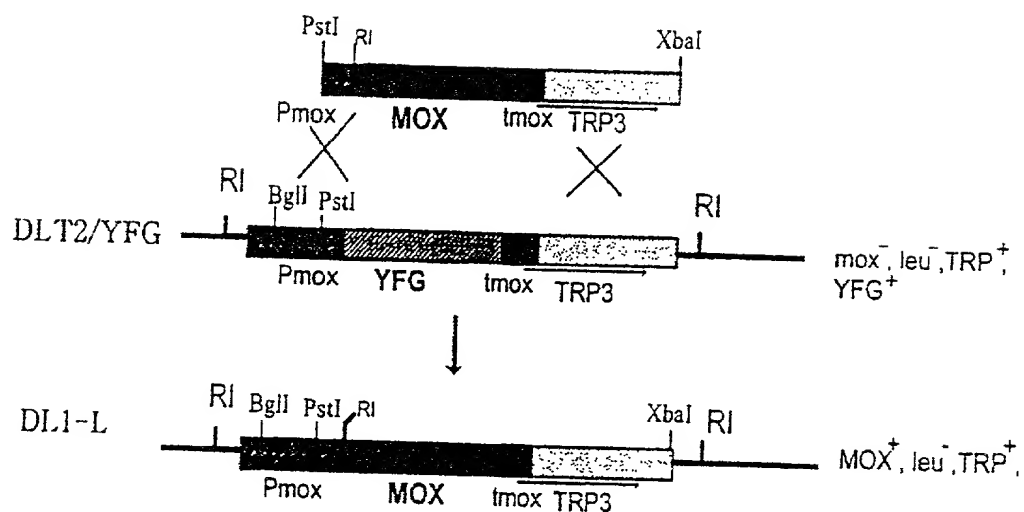
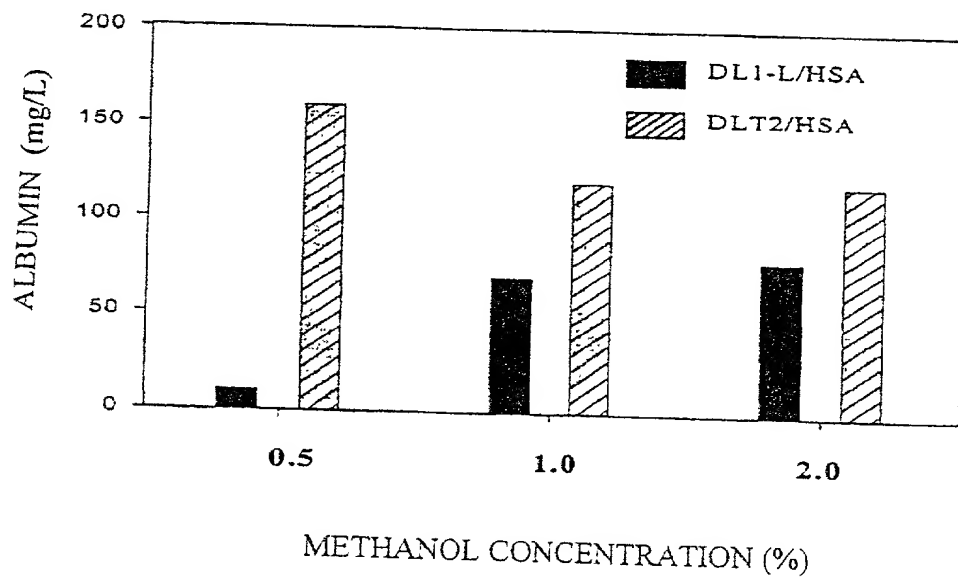


FIG. 13b



12/13

FIG. 13c**FIG. 14**

13/13

FIG. 15

